Data Evaluation Record on the Acute Toxicity of Formulated Flufenacet (36.1% a.i. Flufenacet and 4.4% a.i. Isoxaflutole) to Terrestrial Vascular Plants: Seedling Emergence

PMRA Submission Number {.....} **EPA MRID Number 48897607**

Data Requirement:	PMRA Data Code:	9.8.4 (TGAI) or 9.8.6 (EP)
	EPA DP Barcode:	405834
	OECD Data Point:	IIA 8.12 (TGAI) and IIIA 10.8.1.1 (EP)
	EPA Guideline:	850.4100

Test material: Flufenacet and Isoxaflutole SC 480 formulation

A.I. Flufenacet **Purity:** 36.1%
A.I. Isoxaflutole **Purity:** 4.4%

Common name:
Chemical name:
IUPAC (Flufenacet): 4'-fluoro-N-isopropyl-2-[5-(trifluoromethyl)-1,3,4-thiadiazol-2-yloxy]acetanilide
IUPAC (Isoxaflutole): Not reported
CAS name: Not reported
CAS No.: Not reported
Synonyms: AE F133402 01 SC40 A401

Primary Reviewer: Joan Gaidos **Signature:**
Senior Scientist, CDM Smith **Date:** 7/07/15

Secondary Reviewer: Teri S. Myers **Signature:**
Senior Scientist, CDM Smith **Date:** 7/31/15

Primary Reviewer: Geoffrey Sinclair **Date:** 11/17/15
EPA/OPP/EFED/ERB5

Secondary Reviewer(s): {.....} **Date:** {.....}
{EPA/OECD/PMRA}

Reference/Submission No.: {.....}

Company Code	{.....}	[For PMRA]
Active Code	{.....}	[For PMRA]
Use Site Category:	{.....}	[For PMRA]
EPA PC Code	121903 (Flufenacet)	
	123000 (Isoxaflutole)	

Date Evaluation Completed: {dd-mm-yyyy}

CITATION: Flufenacet and Isoxaflutole SC 480 (AE F133402 01 SC40 A401) Effects on Ten Species of Non-Target Terrestrial Plants: Seedling Emergence and Growth Test (Tier 2). Unpublished study performed and sponsored by Bayer CropScience AG, Frankfurt, Germany. Laboratory Project Number: SE05/059. Study completed April 06, 2006.

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to terrestrial vascular plants. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

Data Evaluation Record on the Acute Toxicity of Formulated Flufenacet (36.1% a.i. Flufenacet and 4.4% a.i. Isoxaflutole) to Terrestrial Vascular Plants: Seedling Emergence

PMRA Submission Number {.....} **EPA MRID Number 48897607**

EXECUTIVE SUMMARY:

The effect of **Flufenacet (36.1% Flufenacet; 4.4% Isoxaflutole)** on the seedling emergence of monocot (oat, *Avena sativa*; ryegrass, *Lolium perenne*; and sorghum, *Sorghum vulgare*) and dicot (cucumber, *Cucumis sativus*; oilseed rape, *Brassica napus*; radish, *Raphanus sativus*; soybean, *Glycine max*; sugar beet, *Beta vulgaris*; sunflower, *Helianthus annuus*; and tomato, *Lycopersicon esculentum*) crops was studied at a nominal concentration of 0 (negative), 0.0023, 0.0042, 0.0084, 0.017, 0.034, and 0.067 lb ai/A for ryegrass, sorghum, sugarbeet, and oats; 0 (negative), 0.0042, 0.0084, 0.017, 0.034, 0.067 and 0.13 lb ai/A for oilseed rape; 0 (negative), 0.0042, 0.0084, 0.017, 0.034, 0.067, 0.13 and 0.27 lb ai/A cucumber and radish; and 0 (negative), 0.017, 0.034, 0.067, 0.13, 0.27 and 0.54 lb ai/A for soybean, sunflower and tomato in terms of Flufenacet. Calculated nominal Isoxaflutole concentrations were 0 (negative control), 0.00028, 0.00051, 0.0010, 0.0020, 0.0041, 0.0081, 0.016, 0.033 and 0.065 lb ai/A.

Flufenacet concentration was analytically confirmed at the highest concentration and was 0.53 lb ai/A. The calculated measured concentration of Isoxaflutole at the highest treatment level was 0.065 lb ai/A.

The growth medium used in the seedling emergence test was standard soil (sandy-silt loam, pH 7.31, organic carbon 1.3%). On day 14 the surviving plants per pot were recorded and dry weight per replicate determined. Height was not determined.

Negative control emergence ranged from 80 to 98%. There were significant inhibitions in emergence for cucumber, ryegrass, sorghum, sugarbeet, and tomato, and not in any other species tested. Cucumber significant inhibitions in emergence compared to the negative control were 21, 27 and 18% at the 0.0084, 0.017 and 0.27 lb ai/A treatment levels, and did not exhibit a dose-response (Mann-Whitney U Two-Sample test, $p<0.05$). Significant inhibitions in sorghum, sugarbeet and tomato were a maximum of 39, 33 and 35%, respectively, compared to the negative control, at the higher treatment levels only. Only ryegrass exhibited a dose response, and significant inhibitions in emergence were 21 and 88% at the 0.034 and 0.067 lb ai/A, respectively compared the negative control (Jonckheere-Terpstra Step-Down test, $p<0.05$).

The reviewer measured survival based on number planted. Negative control survival based on number planted ranged from 78 to 98%. There were significant inhibitions in survival compared to the negative control for all species except oat and soybean and 100% mortality at the higher concentrations in cucumber, oilseed rape, radish, ryegrass, sugarbeet, sunflower and tomato. Significant inhibitions in sugarbeet survival compared to the negative control were 15 to 100% from the 0.0042 to the 0.067 lb ai/A treatment level (Jonckheere-Terpstra Step-Down test, $p<0.05$). Significant inhibitions in radish survival compared to the negative control also showed a dose-response and were 21 to 100% from the 0.017 to the 0.27 lb ai/A treatment level (Jonckheere-Terpstra Step-Down test, $p<0.05$). Significant decreases in ryegrass survival of 38 and 100% and sorghum of 55 and 87% were observed at the 0.034 and 0.067 lb ai/A treatment levels, respectively (Jonckheere-Terpstra Step-Down test and Williams test, respectively; $p<0.05$). Oilseed rape survival significant inhibitions measured 68, 95 and 100% at the 0.034, 0.067 and 0.13 lb ai/A treatment levels, respectively, compared to the negative control (Jonckheere-Terpstra Step-Down test, $p<0.05$). Cucumber and tomato survival exhibited dose response in significant inhibitions in survival of up to 100% starting at the 0.067 lb ai/A treatment level. Significant inhibitions in tomato survival were observed only at the highest two treatment levels and were 100% (Mann-Whitney U Two-Sample test, $p<0.05$).

Significant inhibitions in seedling dry weight were observed in all species except sorghum. Significant inhibitions in ryegrass dry weight measured 31 to 53% from the 0.0042 to the 0.034 lb ai/A treatment level (Williams test, $p<0.05$) and significant inhibitions in radish dry weight measured 60 to 69% over the 0.017 to the 0.067 lb ai/A treatment level (Dunnett's test, $p<0.05$); there was 100% mortality of ryegrass at the highest treatment level and 92 to 100% mortality of radish at the three highest treatment levels. Oilseed rape significant inhibitions in dry weight were 34 to 94% from the 0.0084 to the 0.067 lb ai/A treatment (Williams test, $p<0.05$) and soybean significant inhibitions in dry weight were 18 to 77% from the 0.034 to the 0.53 lb ai/A treatment level (Jonckheere-Terpstra Step-Down test, $p<0.05$) compared to the negative control. Significant inhibitions in tomato did not exhibit dose response and were

57, 74 and 59% at the 0.034, 0.067 and 0.13 lb ai/A treatments, respectively. Significant inhibitions in sugarbeet and sunflower of up to 72 and 68%, respectively, were observed at two higher treatment levels; there was 100% mortality of sugarbeet and sunflower at the highest treatment levels (Williams test, $p<0.05$). Significant inhibitions in cucumber and oat dry weight also exhibited a dose-response and were 40 to 91% and 17 to 51% over the 0.017 to 0.13 lb ai/A and 0.017 to 0.067 lb ai/A treatment range, respectively; there was 100% mortality of cucumber at the highest treatment level, 0.27 lb ai/A (Jonckheere-Terpstra Step-Down test and Williams test, respectively, $p<0.05$).

Seedling height was not measured in the study.

The most sensitive monocot species was ryegrass based on dry weight, with NOAEC and IC_{25} values of 0.0023 and 0.00338 lb ai/A, respectively; and the most sensitive dicot species was radish based on dry weight, with NOAEC and IC_{25} values of 0.0084 and 0.00505 lb ai/A, respectively. The IC_{25} value for radish was less than the NOAEC and may be a result of 92-100% mortality at the three highest treatment levels affecting growth rates and trend analyses.

In terms of the other active ingredient Isoxaflutole, the NOAEC and IC_{25} values for ryegrass based on dry weight were 0.00028 and 0.00041 lb ai/A Isoxaflutole, respectively, and the NOAEC and IC_{25} values for radish based on dry weight were 0.0010 and 0.00062 lb ai/A Isoxaflutole, respectively.

Based on reviewer calculated total formulation concentrations, the NOAEC and IC_{25} values for ryegrass based on dry weight were 0.0064 and 0.0094 lb/A total formulation, respectively, and the NOAEC and IC_{25} values for radish based on dry weight were 0.023 and 0.014 lb/A total formulation, respectively.

The occurrence of phytotoxic effects increased in severity and prevalence with increasing test concentrations; symptoms included wilting, stunting, leaf deformation, bleaching, chlorosis, necrosis, abnormal growth, growth depression, growth suspension and white stem. Maximum effects were D to E (showing poor vigor to moribund) for all species with the exception of sunflower (maximum B=moderate symptoms) and tomato (maximums A=slight symptoms); however, there was mortality >90% at the highest treatment(s) for all species except soybean, oat and sorghum. Effects were dose-related.

Maximum Labeled Rate: Not reported

Results Synopsis

Flufenacet

Monocot

Most sensitive monocot: Ryegrass, based on dry weight

EC_{50}/IC_{50}: 0.0217 lb ai/A	95% C.I.: 0.0138-0.0342 lb ai/A
EC_{25}/IC_{25}: 0.00338 lb ai/A	95% C.I.: 0.00157-0.00643 lb ai/A
EC_{05}/IC_{05}: 0.000232 lb ai/A	95% C.I.: N/A-0.00133 lb ai/A
NOEC: 0.0023 lb ai/A	
Slope: N/A	95% C.I.: N/A

Dicot

Most sensitive dicot: Radish, based on dry weight*

EC_{50}/IC_{50}: 0.0187 lb ai/A	95% C.I.: 0.0121-0.0289 lb ai/A
EC_{25}/IC_{25}: 0.00505 lb ai/A	95% C.I.: 0.00124-0.0126 lb ai/A
EC_{05}/IC_{05}: 0.000767 lb ai/A	95% C.I.: N/A-0.00445 lb ai/A
NOEC: 0.0084 lb ai/A	
Slope: N/A	95% C.I.: N/A

*IC_{25} is less than the NOAEC; growth and the trends analyses may have been affected by 92-100% mortality at the three highest treatment levels.

Isoxaflutole

Monocot

Most sensitive monocot: Ryegrass, based on dry weight

EC_{50}/IC_{50}: 0.0026 lb ai/A 95% C.I.: 0.0017-0.0042 lb ai/A
EC_{25}/IC_{25}: 0.00041 lb ai/A 95% C.I.: 0.00019-0.00078 lb ai/A
EC_{05}/IC_{05}: 0.000028 lb ai/A 95% C.I.: N/A-0.00016 lb ai/A
NOEC: 0.00028 lb ai/A
Slope: N/A 95% C.I.: N/A

Dicot

Most sensitive dicot: Radish, based on dry weight*

EC_{50}/IC_{50}: 0.0023 lb ai/A 95% C.I.: 0.0015-0.0035 lb ai/A
EC_{25}/IC_{25}: 0.00062 lb ai/A 95% C.I.: 0.00015-0.0015 lb ai/A
EC_{05}/IC_{05}: 0.000093 lb ai/A 95% C.I.: N/A-0.00054 lb ai/A
NOEC: 0.00102 lb ai/A
Slope: N/A 95% C.I.: N/A

*IC_{25} is less than the NOAEC; growth and the trends analyses may have been affected by 92-100% mortality at the three highest treatment levels.

Table 1 (Tier II studies). Summary of most sensitive parameters by species (lb ai/A Flufenacet).

Species	Endpoint	NOEC	EC_{05}/IC_{05}	EC_{25}/IC_{25}	EC_{50}/IC_{50}
Cucumber	Dry weight[a]	0.0084	0.00405	0.0123	0.0266
Oat	Dry weight	0.0084	0.00753	0.0269	0.0651
Oilseed Rape	Dry weight[a]	0.0042	0.00183	0.00627	0.0148
Radish	Dry weight[a]	0.0084	0.000767	0.00505*	0.0187
Ryegrass	Dry weight[a]	0.0023	0.000232	0.00338	0.0217
Sorghum	Survival[b]	0.017	0.000462	0.00364	0.0152
Soybean	Dry weight	0.017	0.00305	0.0206	0.0776
Sugarbeet	Survival[a,b]	0.0023	0.00306	0.00638	0.0106
Sunflower	Survival[a,b]	0.13	0.0101	0.0347*	0.0816
Tomato	Dry weight[a]	0.017	0.00151	0.00961*	0.0348

NC – not calculable
* IC_{25} less than the NOAEC for radish and tomato dry weight and sunflower survival. High mortality at the higher treatment levels may have affected the trends analysis.
[a] There was 100% mortality in the highest one or two treatment levels for these species. Low survivorship may have confounded seedling growth effects for these species.
[b] Studies are designed to capture sub-lethal effects, therefore survival is not expected to be the most sensitive endpoint. Low survivorship may have confounded seedling growth effects for these species.

Table 1a (Tier II studies). Summary of most sensitive parameters by species (lb ai/A Isoxaflutole).

Species	Endpoint	NOEC	EC_{05}/IC_{05}	EC_{25}/IC_{25}	EC_{50}/IC_{50}
Cucumber	Dry weight[a]	0.0010	0.00049	0.0015	0.0032
Oat	Dry weight	0.0010	0.00092	0.00328	0.0079
Oilseed Rape	Dry weight	0.00051	0.00022	0.00076	0.0018
Radish	Dry weight[a]	0.0010	0.000093	0.00062*	0.0023
Ryegrass	Dry weight[a]	0.00028	0.000028	0.00041	0.0026

Sorghum	Survival[b]	0.0021	0.000056	0.00044	0.0019
Soybean	Dry weight	0.00207	0.00037	0.00251	0.0095
Sugarbeet	Survival[a,b]	0.00028	0.00037	0.00078	0.0013
Sunflower	Survival[a,b]	0.0158	0.0012	0.0042*	0.0099
Tomato	Dry weight[a]	0.00207	0.00018	0.00143*	0.0042

NC – not calculable

* IC_{25} less than the NOAEC for radish and tomato dry weight and sunflower survival. High mortality at the higher treatment levels may have affected the trends analysis.

[a] There was 100% mortality in the highest one or two treatment levels for these species. Low survivorship may have confounded seedling growth effects for these species.

[b] Studies are designed to capture sub-lethal effects, therefore survival is not expected to be the most sensitive endpoint. The strong effect of low survivorship may have confounded seedling growth effects for these species.

This study is scientifically sound and is classified as supplemental. The most sensitive species, radish, is invalid as is tomato since the IC25 calculated is lower than the lowest concentration tested. Survival is the most sensitive endpoint for sorghum, Sugarbeet, and sunflower which is not expected since the test is designed to examine sublethal effects.

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was conducted in compliance with OPPTS 850.4100: Seedling emergence and seedling growth (January 2012) and OECD 208 Guideline for test of chemicals, Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test (July 2006). The reviewer evaluated the study methods according to EPA Ecological Effects Test Guidelines, OCSPP Guideline 850.4100: Seedling Emergence and Seedling Growth. There were some deficiency and deviations noted by the reviewer.

1. Survival was the most sensitive endpoint for sorghum, sugarbeet and sunflower, with additional significant effects on survival in all other species except oat and soybean. This study is designed to capture sub-lethal effects, therefore survival is not expected to be the most sensitive endpoint and low survivorship may have confounded seedling growth effects in all species except oat and soybean.

2. The IC_{25} for the most sensitive dicot radish, based on dry weight, was less than the NOAEC. Seedling growth and the trends analyses for radish may have been affected by 92 to 100% mortality at the three highest treatment levels. Radish was retained as the most sensitive endpoint for dicots because radish dry weight was significantly inhibited at lower concentrations; significant inhibitions in radish dry weight were over 60% at the 0.017, 0.034 and 0.067 lb ai/A treatment levels. There was 100% mortality of radish at the two highest treatment levels, 0.13 and 0.27 lb ai/A.

3. Three monocots were studied and OCSPP guidelines recommend including four monocots in the study.

4. Mean seedling control survival was 80% for cucumber, 78% for sorghum and 80% for sunflower; OCSPP recommends that mean control seedling survival be at least 90% at test termination.

5. Seedling height was not measured in the study.

6. The physico-chemical properties of the test material were not reported.

7. Soil CEC and % soil moisture were not reported. Water source and volume applied not reported.

8. It was not stated if this study was conducted according to FIFRA Good Laboratory Practice Standards as

published by the U.S. EPA, 40 CFR Part 160 (1989).
The deficiency and deviations did not have an impact on the acceptability of this study.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. This study was conducted in compliance with Good Laboratory Practice Standards as published by OECD Principle of Good Laboratory Practice (C(97)186/final); and German Chemical Act (ChemG), Annex 1, current version.

A. MATERIALS:

1. Test Material **Formulated Flufenacet SC 480 (36.1% a.i. Flufenacet; 4.4% Isoxaflutole)**

Description: Beige milky liquid.

Lot No./Batch No.: EFIM000327

Purity: Flufenacet, 36.1%
Isoxaflutole, 4.4%

Stability of compound under test conditions: Analytical determinations based on measured concentration of the test material in the highest concentration spray solution yielded recoveries of 99.6-100.9% of nominal (n=3). Stability was not determined.

(OECD recommends chemical stability in water and light)

Storage conditions of test chemicals: The test material was stored at room temperature.

Table 2. Physical/chemical properties of Flufenacet

Parameter	Values	Comments
Water solubility at 20˚C	Not reported	
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

2. Test organism:

Monocotyledonous species: Oats (*Avena sativa*, Poaceae, Flamingsstern), Ryegrass (*Lolium perenne*, Poaceae, Deutsches Weidelgras), and sorghum (*Sorghum vulgare*, Poaceae, Piper); *EPA recommends four monocots in two families, including corn.*
Dicotyledonous species: Cucumber (Cucumis sativus; Cucurbitaceae, Delikatess); Oilseed rape (*Brassica napus*, Brassicaceae, Licapo); Radish (*Raphanus sativus*, Brassicaceae, Riesenbutter); Soybean (*Glycine max*, Fabaceae, Erin); Sugar beet (*Beta vulgaris*; Chenopodiaceae, Achat); Sunflower (*Helianthus annuus*, Asteraceae, Big Smile), and Tomato (*Lycopersicon esculentum*, Solanaceae, Balkonstar); *EPA recommends six dicots in four families, including soybean and a root crop.*

OECD recommends a minimum of three species selected for testing, at least one from each of the following categories: Category 1: ryegrass, rice, oat, wheat, and sorghum; Category 2: mustard, rape, radish, turnip, and Chinese cabbage; Category 3: vetch, mung bean, red clover, fenugreek, lettuce, and cress.

Seed source: All seeds supplied by commercial sources.
Prior seed treatment/sterilization: The seeds were not treated with any type of fungicides, insecticides, or any pesticides.
Historical % germination of seed: Historic germination rates were at least 70% (not reported).
Seed storage, if any: Seeds were stored in plastic boxes in the refrigerator.

B. STUDY DESIGN:

1. Experimental Conditions

a. Limit test: None.

b. Range-finding study: None.

c. Definitive Study

Table 3: Experimental Parameters - Seedling Emergence.

Parameters	Seedling Emergence	
	Details	Remarks
		Criteria
Duration of the test	14 days	Test continued for 14 days following the emergence of 70% of control seedlings.
		Recommended test duration is 14-21 days. *OECD recommends that the test be terminated no sooner than 14 days after 50 percent of the control seedlings have emerged*

Parameters	Seedling Emergence	
	Details	**Remarks** ***Criteria***
Number of seeds/plants/species/ replicate	Eight pots (replicates) with 5 seeds per pot.	*Ten seeds per replicate should be used.* *OECD recommends a minimum of five seeds planted in each replicate within 24 hours of incorporation of the test substance. All seeds of each species for each test should be of the same size class. The seed should not be imbibed.*
Number of replicates Control: Adjuvant control: Treated:	8 N/A 8	*Four replicates per dose should be used.* *OECD recommends a minimum of four replicates per treatment*
Test concentrations (lb ai/A) Nominal: Measured:	**Flufenacet** 0 (negative), 0.0023, 0.0042, 0.0084, 0.017, 0.034, and 0.067 lb ai/A for ryegrass, sorghum, sugarbeet, and oats; 0 (negative), 0.0042, 0.0084, 0.017, 0.034, 0.067, and 0.13 lb ai/A for oilseed rape; 0 (negative), 0.0084, 0.017, 0.034, 0.067, 0.13 and 0.27 lb ai/A cucumber and radish; 0 (negative), 0.017, 0.034, 0.067, 0.13, 0.27 and 0.54 lb ai/A for soybean, sunflower and tomato. Highest concentration of Flufenacet was measured: **Flufenacet**: 0.53 lb ai/A **Isoxaflutole (calculated)**: 0.065 lb ai/A	Nominal isoxaflutole concentrations of 0 (negative), 0.00028, 0.00051, 0.0010, 0.0020, 0.0041, 0.0081, 0.016, 0.033 and 0.065 lb ai/A *Five test concentrations should be used with a dose range of 2X or 3X progression* *OECD recommends three concentrations, preferably with application rates equivalent to 0.0 (control), 1.0, 10.0 and 100 mg substance per kg of oven-dried soil.*
Method and interval of analytical verification	Duplicate samples were taken from the negative control and the treatment groups for analysis via HPLC with LiChrosorb RP Select B column with UV detection (230 nm).	

Parameters	Seedling Emergence	
	Details	Remarks
		Criteria
LOQ: LOD:	N/A Not reported.	
Adjuvant (type, percentage, if used)	N/A	
Test container (pot) Size/Volume Material: (glass/polystyrene)	Pots with diameter of 10 cm. Plastic	*Non-porous containers should be used.* *OECD recommends that non-porous plastic or glazed pot be used.*
Growth facility	Greenhouse	
Method/depth of seeding	Seeds introduced manually to the soil surface and covered with 0.2 to 0.6 cm layer of soil:quartz sand (1:1 mix) and soil top watered immediately.	
Test material application Application time including the plant growth stage Number of application Application interval Method of application	 After planting. 1 N/A- single application The test material was applied using a linear cabinet track sprayer (SprayLab SLGH 2500) with a Teejet 8001 EVSV nozzle, applied 30 cm from the soil surface.	
Details of soil used Geographic location Depth of soil collection Soil texture % sand	 N/A N/A Sandy-silt loam 59.1	Standard soil sieved to 2 mm and sterilized.

Parameters	Seedling Emergence	
	Details	**Remarks**
		Criteria
% silt % clay pH: % organic carbon CEC Moisture at 1/3 atm (%)	24.2 16.7 7.31 1.3 Not reported Not reported	*Soil mixes containing sandy loam, loam, or clay loam soil with no greater than 2% organic matter are preferable. Glass beads, rock wool, and 100% acid washed sand are not preferred.* *OECD prefers the soil to be sieved (0.5 cm) to remove coarse fragments. Carbon content should not exceed 1.5% (3% organic matter). Fine particles (under 20um) makeup should be between 10 and 20%. The recommended pH is between 5.0 and 7.5.*
Details of nutrient medium, if used	N/A	
<u>Watering regime and schedules</u> Water source/type: Volume applied: Interval of application: Method of application:	Not reported. Not reported. Daily. The plants were bottom watered daily.	 *EPA prefers that bottom watering be utilized for seedling emergence studies so that the chemical is not leached out of the soil during the test.*
Any pest control method/fertilization, if used	2.4 g Blaukorn/L added to bulk soil.	
<u>Test conditions</u> Temperature: Photoperiod: Light intensity and quality: Relative humidity:	 Day: 23±8˚C; Night 18±8˚C 16L:8D Natural sunlight supplemented with artificial light. >15000 and <20000 Lux 31-98%	Actual ranges of specific test conditions were not reported. *EPA prefers that the cold vs warm loving plants be tested in two separate groups to optimize plant growth.* *OECD prefers that the temperature, humidity and light conditions be suitable for maintaining normal growth of each species for the test period.*
<u>Reference chemical (if used)</u> Name: Concentrations:	N/A	
Other parameters, if any	None	

2. Observations:

Table 4: Observation Parameters - Seedling Emergence.

Parameters	Seedling Emergence	
	Details	**Remarks**
Parameters measured (e.g., number of germinated seeds, emerged seedlings, plant height, dry weight or other endpoints)	- Emergence - Survival - Dry weight - Phytotoxicity	
Measurement technique for each parameter	Emergence and phytotoxicity were visually determined. Survival was defined as the percent of emerged by the study author. Mean plant weight was estimated measuring the Mean dry weight per replicate following oven drying.	
Observation intervals	Each pot was inspected weekly, emergence and phytotoxicity assessments performed. Dry weight was recorded at study termination.	
Other observations, if any	N/A	
Were raw data included?	Yes	
Phytotoxicity rating system, if used	0=No injury or effect; A=Slight symptoms throughout the whole plant or more moderate symptoms on a small area (i.e. one leaf); B=Moderate symptoms throughout the whole plant or severe symptoms on limited area, (i.e.one-two leaves); C=Severe symptoms throughout whole plant with younger or newly developed leaves growing normally; D=Total plant symptoms with the plant showing poor vigor; E=Moribund.	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

1. Seedling Emergence:

The study author reported negative control emergence ranged from 83 to 98%. The study author found significant inhibitions in emergence in ryegrass, sorghum, sugarbeet and tomato at the highest one or two treatment levels only, and not in any other species tested. Ryegrass, sorghum, sugarbeet and tomato significant lack of emergence were a maximum 90, 50, 35 and 50%, respectively (Fisher's Exact test with Bonferroni Correction, $p<0.05$). The reviewer found significant inhibitions in emergence in cucumber, in addition to significant inhibitions in ryegrass, sorghum, sugarbeet and tomato. Cucumber significant inhibitions in emergence compared to the negative control were 21, 27 and 18% at the 0.0084, 0.017 and 0.27 lb ai/A treatment levels, and did not exhibit a dose-response (Mann-Whitney U Two-Sample test, $p<0.05$). Significant inhibitions in sorghum, sugarbeet and tomato were a maximum of 39, 33 and 35%, respectively, compared to the negative control, at the higher treatment levels only. Only ryegrass exhibited a dose response, and significant inhibitions in emergence were 21 and 88% at the 0.034 and 0.067 lb ai/A, respectively compared the negative control (Jonckheere-Terpstra Step-Down test, $p<0.05$).

The study author reported % survived based on number emerged. The study author reported negative control survival was 100% for all species, except sorghum that had a negative control survival of 94%. The study author found significant inhibitions in survival for all species except oat and soybean. There were significant decreases in survival of maximums of 100% in cucumber, oilseed rape, radish, ryegrass, sugarbeet, sunflower and tomato at treatment levels between 0.017 and 0.54 lb ai/A (Fisher's Exact test with Bonferroni Correction, $p<0.05$). Significant decreases in sorghum survival were a maximum of 80% at the 0.067 lb ai/A treatment level A (Fisher's Exact test with Bonferroni Correction, $p<0.05$).

The reviewer measured survival based on number planted. Negative control survival based on number planted ranged from 78 to 98%. The reviewer also found significant inhibitions in survival compared to the negative control for all species except oat and soybean. Significant inhibitions in sugarbeet survival compared to the negative control were 15 to 100% from the 0.0042 to the 0.067 lb ai/A treatment level (Jonckheere-Terpstra Step-Down test, $p<0.05$). Significant inhibitions in radish survival compared to the negative control also showed a dose-response and were 21 to 100% from the 0.017 to the 0.27 lb ai/A treatment level (Jonckheere-Terpstra Step-Down test, $p<0.05$). Significant decreases in ryegrass survival were 38 and 100% and significant decreases in sorghum survival were 55 and 87% at the 0.034 and 0.067 lb ai/A treatment levels, respectively, compared to the negative control (Jonckheere-Terpstra Step-Down test and Williams test, respectively; $p<0.05$). Oilseed rape survival significant inhibitions measured 68, 95 and 100% at the 0.034, 0.067 and 0.13 lb ai/A treatment levels, respectively, compared to the negative control (Jonckheere-Terpstra Step-Down test, $p<0.05$). Cucumber and tomato survival exhibited dose response in significant inhibitions in survival of up to 100% starting at the 0.067 lb ai/A treatment level. Significant inhibitions in tomato survival were observed only at the highest two treatment levels and were 100% (Mann-Whitney U Two-Sample test, $p<0.05$).

The study author found inhibitions in seedling dry weight in all species except sorghum. Significant inhibitions in ryegrass dry weight measured 31 to 53% from the 0.0042 to the 0.034 lb ai/A treatment level (Williams multiple sequential t-test, $p<0.05$) and significant inhibitions in radish dry weight measured 60 to 69% from the 0.017 to the 0.067 lb ai/A treatment level (Dunnett's multiple t-test, $p<0.05$); there was 100% mortality of ryegrass at the highest treatment level and 100% mortality of radish at the two highest treatment levels. Oilseed rape significant inhibitions in dry weight were 34 to 94% from the 0.0084 to the 0.067 lb ai/A treatment and soybean significant inhibitions in dry weight were 18 to 77% from the 0.034 to the 0.54 lb ai/A compared to the negative control (Williams multiple sequential t-test, $p<0.05$). Significant

inhibitions in tomato did not exhibit dose response and were 57, 74 and 59% at the 0.034, 0.067 and 0.13 lb ai/A treatment, respectively. Significant inhibitions in oat, sugarbeet and sunflower of up to 51, 72 and 68%, respectively, were observed at the higher treatment levels (Dunnett's multiple t-test, $p<0.05$); there was 100% mortality in sugarbeet and sunflower at the highest treatment levels. The reviewer found similar significant inhibitions as the study author in cucumber, oilseed rape, radish, ryegrass, soybean, sugarbeet, sunflower and tomato. The reviewer also measured significant inhibitions in oat of 17% at a lower treatment level, 0.017 lb ai/A (Williams test, $p<0.05$).

Seedling height was not measured in the study.

The study author based the most sensitive species on the EC/IC_{50} and not the EC/IC_{25}. The study authors most sensitive monocot species was ryegrass based on dry weight, with NOAEC, IC_{25} and IC_{50} values of 0.0023, 0.0039 and 0.022 lb ai/A, respectively. Based on the EC_{50}, the study author's most sensitive dicot species was sugarbeet based on survival, with NOAEC and EC_{50} values of 0.0023 and 0.014 lb ai/A, respectively, but based on the EC/IC25 the study author's most sensitive dicot was radish based on dry weight with NOAEC and IC_{25} values of 0.0084 and 0.0059 lb ai/A, respectively.

The occurrence of phytotoxic effects increased in severity and prevalence with increasing test concentrations; symptoms included wilting, stunting, leaf deformation, bleaching, chlorosis, necrosis, abnormal growth, growth depression, growth suspension and white stem. Maximum effects were D to E (showing poor vigor to moribund) for all species with the exception of sunflower (maximum B=moderate symptoms) and tomato (maximums A=slight symptoms); however, there was still mortality >90% at the highest treatment(s) for all species except soybean, oat and sorghum. Effects were dose-related.

B. REPORTED STATISTICS:

Emergence, survival, dry weight, and height means and standard deviations were determined for control and treatment groups. Treatments were compared to the negative control. NOEC and LOEC, and EC_{25} and EC_{50} determined using ToxRat software (ToxRatPro, version 2.09, release 27.01.2005). All statistical determinations were made with 95% certainty. Nominal concentrations were used for all analyses.

Table 5: Effect of Flufenacet and Isoxaflutole on 14-Day Seedling Emergence

Species	**Results summary for height (lb ai/A Flufenacet)**									
	height (cm)	NOEC	IC_{05}	95%CI	IC_{25}	95%CI	IC_{50}	95%CI	slope	95%CI
Cucumber	ND	ND	ND	N/A	ND	N/A	ND	N/A	N/A	N/A
Oat	ND	ND	ND	N/A	ND	N/A	ND	N/A	N/A	N/A
Oilseed Rape	ND	ND	ND	N/A	ND	N/A	ND	N/A	N/A	N/A
Radish	ND	ND	ND	N/A	ND	N/A	ND	N/A	N/A	N/A
Ryegrass	ND	ND	ND	N/A	ND	N/A	ND	N/A	N/A	N/A
Sorghum	ND	ND	ND	N/A	ND	N/A	ND	N/A	N/A	N/A
Soybean	ND	ND	ND	N/A	ND	N/A	ND	N/A	N/A	N/A
Sugarbeet	ND	ND	ND	N/A	ND	N/A	ND	N/A	N/A	N/A
Sunflower	ND	ND	ND	N/A	ND	N/A	ND	N/A	N/A	N/A
Tomato	ND	ND	ND	N/A	ND	N/A	ND	N/A	N/A	N/A

ND- Not determined. NC- Not calculable.

Table 5a: Effect of Flufenacet and Isoxaflutole on 14-Day Seedling Emergence

Species	Results summary for biomass (lb ai/A Flufenacet)*									
	weight (g)	NOEC	IC_{05}	95%CI	IC_{25}	95%CI	IC_{50}	95%CI	slope	95%CI
Cucumber[1]	0.0372-0.418	0.0084	ND	N/A	0.014	0.0045-0.021	0.027	0.017-0.043	N/A	N/A
Oat[2]	0.0624-0.126	0.017	ND	N/A	0.025	0.016-0.032	0.063	0.047-0.10	N/A	N/A
Oilseed Rape[3]	0.014-0.209	0.0042	ND	N/A	0.0067	0.0038-0.0092	0.015	0.011-0.019	N/A	N/A
Radish[4]	0.062-0.197	0.0084	ND	N/A	0.0059	ND	0.021	ND	N/A	N/A
Ryegrass[5]	0.0189-0.0397	0.0023	ND	N/A	0.0039	0.00015-0.0078	0.022	0.011-0.20	N/A	N/A
Sorghum	0.024-0.047	0.067	ND	N/A	NC	N/A	>0.067	N/A	N/A	N/A
Soybean[6]	0.0593-0.255	<0.017	ND	N/A	0.031	0.0053-0.057	0.087	0.044-0.17	N/A	N/A
Sugarbeet[7]	0.023-0.0826	0.0084	ND	N/A	0.0077	0-0.014	0.016	0.0056-0.10	N/A	N/A
Sunflower[8]	0.0564-0.175	0.034	ND	N/A	0.048	ND	0.085	ND	N/A	N/A
Tomato[9]	0.018-0.0703	0.017	ND	N/A	0.010	ND	0.039	ND	N/A	N/A

ND- Not determined. NC- Not calculable.

*All statistically significant effects analysed by either Fisher's Exact Binomial Test with Bonferroni Correction, Williams multiple sequential t-test, or Dunnett's multiple t-test, $p<0.05$ (not specified).

[1] Significant decrease in cucumber weight, inhibition of 40, 56, 78 and 91% at the 0.017, 0.034, 0.067 and 0.13 lb ai/A treatments, respectively, compared to the negative control (Williams multiple sequential t-test, $p<0.05$).

[2] Significant decrease in oat weight, inhibitions of 35 and 51% at the 0.034 and 0.067 lb ai/A treatments, respectively, compared to the negative control (Dunnett's Multiple t-test, $p<0.05$).

[3] Significant decrease in oilseed rape weight, inhibition of 34, 56, 70 and 94% at the 0.0084, 0.017, 0.034 and 0.067 lb ai/A treatments, respectively, compared to the negative control (Williams multiple sequential t-test, $p<0.05$).

[4] Significant decrease in radish weight, inhibition of 69, 60 and 63% at the 0.017, 0.034 and 0.067 lb ai/A treatments, respectively, compared to the negative control (Dunnett's Multiple t-test, $p<0.05$).

[5] Significant decrease in ryegrass weight, inhibitions of 31, 37, 49 and 53% at the 0.0042, 0.0084, 0.017 and 0.034 lb ai/A treatments, respectively, compared to the negative control (Williams multiple sequential t-test, $p<0.05$).

[6] Significant decrease in soybean weight, inhibition of 18 to 77% beginning at the 0.034 lb ai/A treatment compared to the negative control (Williams multiple sequential t-test, $p<0.05$).

[7] Significant decrease in sugarbeet weight, inhibitions of 63 and 72% at the 0.017 and 0.034 lb ai/A treatments, respectively, compared to the negative control (Dunnett's Multiple t-test, $p<0.05$).

[8] Significant decrease in sunflower weight, inhibition of 45 and 68% at the 0.067 and 0.13 lb ai/A treatments, respectively, compared to the negative control (Dunnett's Multiple t-test, $p<0.05$).

[9] Significant decrease in tomato weight, inhibition of 57, 74 and 59% at the 0.034, 0.067 and 0.13 lb ai/A treatments compared to the negative control (Dunnett's Multiple t-test, $p<0.05$).

Table 5b: Effect of Flufenacet and Isoxaflutole on 14-Day Seedling Emergence

Species	**Results summary for emergence (lb ai/A Flufenacet)****									
	%	NOEC	EC_{05}	95%CI	EC_{25}	95%CI	EC_{50}	95%CI	slope	95%CI
Cucumber	63-85	0.27	ND	N/A	>0.27	N/A	>0.27	N/A	N/A	N/A
Oat	85-95	0.067	ND	N/A	>0.067	N/A	>0.067	N/A	N/A	N/A
Oilseed Rape	85-95	0.13	ND	N/A	>0.13	N/A	>0.13	N/A	N/A	N/A
Radish	95-100	0.27	ND	N/A	>0.27	N/A	>0.27	N/A	N/A	N/A
Ryegrass[1]	10-95	0.034	ND	N/A	0.035	0.028-0.043	0.044	0.038-0.051	N/A	N/A
Sorghum[2]	50-83	0.034	ND	N/A	0.053	0.046-0.072	0.079	0.052-0.12	N/A	N/A
Soybean	88-98	0.53	ND	N/A	>0.53	N/A	>0.53	N/A	N/A	N/A
Sugarbeet[3]	65-98	0.017	ND	N/A	0.054	ND	>0.067	N/A	N/A	N/A
Sunflower	68-88	0.53	ND	N/A	>0.53	N/A	>0.53	N/A	N/A	N/A
Tomato[4]	55-90	0.13	ND	N/A	0.27	0.16-0.45	>0.53	N/A	N/A	N/A

ND- Not determined. NC- Not calculable.
* Inhibitions compared to the control were not quantified.
**Statistically significant effects analysed by Fisher's Exact test with Bonferroni Correction, $p<0.05$.
[1] Significant decrease in ryegrass emergence, inhibition at the 0.067 lb ai/A treatments compared to the negative control.
[2] Significant decrease in sorghum emergence, inhibition at the 0.067 lb ai/A treatments compared to the negative control.
[3] Significant decrease in sugarbeet emergence, inhibition at the 0.034 lb ai/A treatments compared to the negative control.
[4] Significant decrease in tomato emergence, inhibition at the 0.27 and 0.54 lb ai/A treatments compared to the negative control.

Table 5c: Effect of Flufenacet and Isoxaflutole on 14-Day Seedling Emergence

Species	**Results summary for survival (lb ai/A Flufenacet); based on # emerged****									
	%	NOEC	EC_{05}	95%CI	EC_{25}	95%CI	EC_{50}	95%CI	Slope*	95%CI
Cucumber[1]	0-100	0.0084	ND	N/A	0.065	0.024-0.10	0.10	0.056-0.18	N/A	N/A
Oat	86-100	0.067	ND	N/A	>0.067	N/A	>0.067	N/A	N/A	N/A
Oilseed Rape[2]	0-100	0.0084	ND	N/A	0.021	0.018-0.025	0.029	0.025-0.033	N/A	N/A
Radish[3]	0-100	0.0084	ND	N/A	0.024	0.014-0.032	0.035	0.024-0.049	N/A	N/A
Ryegrass[4]	0-100	0.017	ND	N/A	0.033	ND	0.046	ND	N/A	N/A
Sorghum[5]	20-94	0.017	ND	N/A	0.022	0-0.038	0.035	0.012-3.61	N/A	N/A
Soybean	100	0.53	ND	N/A	>0.53	N/A	>0.53	N/A	N/A	N/A
Sugarbeet[6]	0-100	0.0023	ND	N/A	0.0092	0.0076-0.011	0.014	0.012-0.016	N/A	N/A
Sunflower[7]	0-100	0.067	ND	N/A	0.12	0.082-0.16	0.15	0.12-0.23	N/A	N/A
Tomato[8]	0-100	0.034	ND	N/A	0.049	0.041-0.058	0.068	0.058-0.079	N/A	N/A

ND- Not determined. NC- Not calculable.

* Inhibitions compared to the control were not quantified.

**Statistically significant effects analysed by Fisher's Exact test with Bonferroni Correction, $p<0.05$.

[1] Significant decrease in cucumber survival, inhibition at the 0.017, 0.034, 0.067, 0.13 and 0.27 lb ai/A treatments, respectively, compared to the negative control.

[2] Significant decrease in oilseed rape survival, inhibition at the 0.017, 0.034, 0.067 and 0.13 lb ai/A treatments, respectively, compared to the negative control.

[3] Significant decrease in radish survival, inhibition at the 0.017, 0.034, 0.067, 0.13 and 0.27 lb ai/A treatments, respectively, compared to the negative control.

[4] Significant decrease in ryegrass survival, inhibition at the 0.034 and 0.067 lb ai/A treatments, respectively, compared to the negative control.

[5] Significant decrease in sorghum survival, inhibition at the 0.034 and 0.067 lb ai/A treatments, respectively, compared to the negative control.

[6] Significant decrease in sugarbeet survival, inhibition beginning at the 0.0042 lb ai/A treatments compared to the negative control.

[7] Significant decrease in sunflower survival, inhibition at the 0.13, 0.27 and 0.54 lb ai/A treatments, respectively, compared to the negative control.

[8] Significant decrease in tomato survival, inhibition at the 0.067, 0.13, 0.27 and 0.54 lb ai/A treatments, respectively, compared to the negative control.

Mid-study emergence											
Control	Cucumber	Oat	Oilseed Rape	Radish	Ryegrass	Sorghum	Soybean	Sugarbeet	Sunflower	Tomato	Formulation Blank
Not reported											N/A

Plant Injury Index*											
Control	Cucumber	Oat	Oilseed Rape	Radish	Ryegrass	Sorghum	Soybean	Sugarbeet	Sunflower	Tomato	Formulation Blank
0	0-E*	0-D	0-E*	0-E*	0-E*	0	0-D	0-E*	0-B*	0-A*	N/A

0=No injury or effect; A=Slight symptoms throughout the whole plant or more moderate symptoms on a small area (i.e. one leaf); B=Moderate symptoms throughout the whole plant or severe symptoms on limited area, (i.e.one-two leaves); C=Severe symptoms throughout whole plant with younger or newly developed leaves growing normally; D=Total plant symptoms with the plant showing poor vigor; E=Moribund.
*No data at the highest treatment level(s) due to >90% mortality.

C. VERIFICATION OF STATISTICAL RESULTS BY THE REVIEWER:

All analyses were conducted comparing treated to the negative control. These analyses were conducted using CETIS version 1.8.7.12 and backend settings approved for use by EFED on 3/25/14. Data for each endpoint were tested to determine if their distributions were normal and if their variances were homogeneous using Shapiro-Wilk's and Levene's tests, respectively. Data that satisfied these assumptions were subjected to Dunnett's and William's tests, and data that did not satisfy these assumptions were subjected to the non-parametric Mann-Whitney U and Jonckheere's tests. The measured concentration of Flufenacet was available for the highest treatment level; nominal concentrations were used for all other analyses. Linear (survival and emergence) and nonlinear (height and dry weight) regression models were used to interpret EC/ICx values.

Table 6: Effect of Flufenacet and Isoxaflutole on 14-Day Seedling Emergence

Species	**Results summary for height (lb ai/A Flufenacet); Not measured**									
	height (cm)	NOEC	IC_{05}	95%CI	IC_{25}	95%CI	IC_{50}	95%CI	slope	95%CI
Cucumber	ND	ND	ND	N/A	ND	N/A	ND	N/A	N/A	N/A
Oat	ND	ND	ND	N/A	ND	N/A	ND	N/A	N/A	N/A
Oilseed Rape	ND	ND	ND	N/A	ND	N/A	ND	N/A	N/A	N/A
Radish	ND	ND	ND	N/A	ND	N/A	ND	N/A	N/A	N/A
Ryegrass	ND	ND	ND	N/A	ND	N/A	ND	N/A	N/A	N/A
Sorghum	ND	ND	ND	N/A	ND	N/A	ND	N/A	N/A	N/A
Soybean	ND	ND	ND	N/A	ND	N/A	ND	N/A	N/A	N/A
Sugarbeet	ND	ND	ND	N/A	ND	N/A	ND	N/A	N/A	N/A
Sunflower	ND	ND	ND	N/A	ND	N/A	ND	N/A	N/A	N/A
Tomato	ND	ND	ND	N/A	ND	N/A	ND	N/A	N/A	N/A

ND- Not determined. NC- Not calculable.

Table 6a: Effect of Flufenacet and Isoxaflutole on 14-Day Seedling Emergence

Species	Results summary for biomass (lb ai/A Flufenacet)									
	weight (g)	NOEC	IC_{05}	95%CI	IC_{25}	95%CI	IC_{50}	95%CI	slope	95%CI
Cucumber[1]	0.0372-0.418	0.0084	0.00405	N/A-0.0086	0.0123	0.00837-0.0168	0.0266	0.0215-0.0328	N/A	N/A
Oat[2]	0.0624-0.126	0.0084	0.00753	N/A-0.0133	0.0269	0.0188-0.0362	0.0651	0.049-0.0864	N/A	N/A
Oilseed Rape[3]	0.014-0.209	0.0042	0.00183	N/A-0.00396	0.00627	0.00425-0.00865	0.0148	0.012-0.0182	N/A	N/A
Radish[4]	0.062-0.197	0.0084	0.000767	N/A-0.00445	0.00505	0.00124-0.0126	0.0187	0.0121-0.0289	N/A	N/A
Ryegrass[5]	0.0189-0.0397	0.0023	0.000232	N/A-0.00133	0.00338	0.00157-0.00643	0.0217	0.0138-0.0342	N/A	N/A
Sorghum	0.024-0.047	0.067	0.00349	N/A-0.019	0.0313	0.00764-0.0824	0.144	0.00255-8.09*	N/A	N/A
Soybean[6]	0.0593-0.255	0.017	0.00305	N/A-0.00838	0.0206	0.014-0.0289	0.0776	0.0637-0.0946	N/A	N/A
Sugarbeet[7]	0.023-0.0826	0.0084	0.00389	N/A-0.0068	0.00928	0.00569-0.0132	0.017	0.0127-0.0226	N/A	N/A
Sunflower[8]	0.0564-0.175	0.034	0.023	N/A-0.0342	0.0508	0.0388-0.0632	0.0882	0.0768-0.101	N/A	N/A
Tomato[9]	0.018-0.0703	0.017	0.00151	N/A-0.0117	0.00961	0.000901-0.0305	0.0348	0.0194-0.0623	N/A	N/A

ND- Not determined. NC- Not calculable.

*Endpoints and/or confidence limits are outside the tested range of concentrations and should be interpreted cautiously

[1] Significant decrease in cucumber weight, inhibition of 40, 56, 78 and 91% at the 0.017, 0.034, 0.067 and 0.13 lb ai/A treatments, respectively, compared to the negative control (Jonckheere-Terpstra Step-Down test, $p<0.05$). There was 100% mortality of cucumber at the 0.27 lb ai/A treatment.

[2] Significant decrease in oat weight, inhibitions of 17, 35 and 51% at the 0.017, 0.034 and 0.067 lb ai/A treatments, respectively, compared to the negative control (Williams test, $p<0.05$).

[3] Significant decrease in oilseed rape weight, inhibition of 34, 56, 70 and 93% at the 0.0084, 0.017, 0.034 and 0.067 lb ai/A treatments, respectively, compared to the negative control (Williams test, $p<0.05$). There was 100% mortality of oilseed at the highest treatment level, 0.067 lb ai/A.

[4] Significant decrease in radish weight, inhibition of 69, 60 and 63% at the 0.017, 0.034 and 0.067 lb ai/A treatments, respectively, compared to the negative control (Dunnett's test, $p<0.05$). There was 100% mortality of radish at the two highest treatment levels, 0.13 and 0.27 lb ai/A.

[5] Significant decrease in ryegrass weight, inhibitions of 31, 38, 49 and 53% at the 0.0042, 0.0084, 0.017 and 0.034 lb ai/A treatments, respectively, compared to the negative control (Williams test, $p<0.05$). There was 100% mortality of ryegrass at the highest treatment level, 0.067 lb ai/A.

[6] Significant decrease in soybean weight, inhibition of 18 to 77% from the 0.034 to the 0.53 lb ai/A treatments compared to the negative control (Jonckheere-Terpstra Step-Down test, $p<0.05$).

[7] Significant decrease in sugarbeet weight, inhibitions of 63 and 72% at the 0.017 and 0.034 lb ai/A treatments, respectively, compared to the negative control (Williams test, $p<0.05$). There was 100% mortality of sugarbeet at the highest treatment level, 0.067 lb ai/A.

[8] Significant decrease in sunflower weight, inhibition of 45 and 68% at the 0.067 and 0.13 lb ai/A treatments, respectively, compared to the negative control (Williams test, $p<0.05$). There was 100% mortality of sunflower at the two highest treatment levels, 0.27 and 0.53 lb ai/A.

[9] Significant decrease in tomato weight, inhibition of 59, 74 and 59% at the 0.034, 0.067 and 0.13 lb ai/A treatments, respectively, compared to the negative control (Dunnett's test, $p<0.05$). There was 100% mortality of tomato at the two highest treatment levels, 0.27 and 0.53 lb ai/A.

Table 6b: Effect of Flufenacet and Isoxaflutole on 14-Day Seedling Emergence

Species	**Results summary for emergence (lb ai/A Flufenacet)**									
	%	NOEC	EC_{05}	95%CI	EC_{25}	95%CI	EC_{50}	95%CI	slope	95%CI
Cucumber[1]	63-85	<0.0084	NC	N/A	NC	N/A	NC	N/A	N/A	N/A
Oat	85-95	0.067	>0.067	N/A	>0.067	N/A	>0.067	N/A	N/A	N/A
Oilseed Rape	85-95	0.13	NC	N/A	>0.13	N/A	>0.13	N/A	N/A	N/A
Radish	95-100	0.27	>0.27	N/A	>0.27	N/A	>0.27	N/A	N/A	N/A
Ryegrass[2]	10-95	0.017	0.00252	0.000694-0.00476	0.0114	0.00665-0.0171	0.0326	0.0217-0.0612	1.48	0.914-2.04
Sorghum[3]	50-83	0.034	0.000205	N/A-0.00159	0.0134	0.00213-0.0653	0.244	0.0556-306000*	0.535	0.0912-0.978
Soybean	88-98	0.53	NC	N/A	>0.53	N/A	>0.53	N/A	N/A	N/A
Sugarbeet[4]	65-98	0.017	0.00116	$1.01x10^{-5}$-0.00373	0.0431	0.0206-0.351	0.532	0.121-280*	0.618	0.225-1.01
Sunflower	68-88	0.53	NC	N/A	>0.53	N/A	>0.53	N/A	N/A	N/A
Tomato[5]	55-90	0.13	0.00691	0.000715-0.018	0.0998	0.055-0.165	0.638	0.332-2.63*	0.837	0.476-1.2

ND- Not determined. NC- Not calculable.

*Endpoints and/or confidence limits are outside the tested range of concentrations and should be interpreted cautiously

[1] Significant decrease in cucumber emergence, inhibition of 21, 27 and 18% at the 0.0084, 0.017 and 0.27 lb ai/A treatments, respectively, compared to the negative control (Mann-Whitney U Two-Sample test, $p<0.05$); not a dose response.

[2] Significant decrease in ryegrass emergence, inhibition of 21 and 88% at the 0.034 and 0.067 lb ai/A treatments compared to the negative control (Jonckheere-Terpstra Step-Down test, $p<0.05$).

[3] Significant decrease in sorghum emergence, inhibition of 39% at the 0.067 lb ai/A treatments compared to the negative control (Mann-Whitney U Two-Sample test, $p<0.05$).

[4] Significant decrease in sugarbeet emergence, inhibition of 33 and 18% at the 0.034 and 0.067 lb ai/A treatments, respectively, compared to the negative control (Mann-Whitney U Two-Sample test, $p<0.05$).

[5] Significant decrease in tomato emergence, inhibition of 35 and 35% at the 0.27 and 0.53 lb ai/A treatments, respectively, compared to the negative control (Dunnett's test, $p<0.05$).

Table 6c: Effect of Flufenacet and Isoxaflutole on 14-Day Seedling Emergence

Species	Results summary for survival (lb ai/A Flufenacet); based on # planted									
	%	NOEC	EC_{05}	95%CI	EC_{25}	95%CI	EC_{50}	95%CI	slope	95%CI
Cucumber[1]	0-80	0.034	0.00232	0.000497-0.00518	0.0123	0.00564-0.0194	0.0392	0.0261-0.057	1.34	0.893-1.78
Oat	75-93	0.067	0.00074	N/A-0.00386	0.127	0.0342-$1x10^{10}$*	4.53*	0.261-$1x10^{10}$*	0.434	0.0312-0.837
Oilseed Rape[2]	0-93	0.017	0.00608	0.00364-0.00846	0.0132	0.00976-0.0166	0.0227	0.0182-0.0283	2.87	2.15-3.6
Radish[3]	0-100	0.0084	0.0126	0.00905-0.0158	0.0224	0.0183-0.0262	0.0332	0.0286-0.0386	3.92	3.03-4.81
Ryegrass[4]	0-95	0.017	0.00289	0.00106-0.00495	0.01	0.00617-0.0142	0.0238	0.0169-0.0368	1.8	1.18-2.41
Sorghum[5]	10-78	0.017	0.000462	$1.84x10^{-5}$-0.00149	0.00364	0.000899-0.00678	0.0152	0.00856-0.0305	1.08	0.556-1.61
Soybean	88-98	0.53	NC	N/A	>0.53	N/A	>0.53	N/A	N/A	N/A
Sugarbeet[6]	0-98	0.0023	0.00306	0.00211-0.00398	0.00638	0.0051-0.00763	0.0106	0.00895-0.0126	3.05	2.42-3.67
Sunflower[7]	0-80	0.13	0.0101	0.004-0.0174	0.0347	0.021-0.0486	0.0816	0.0596-0.11	1.81	1.3-2.33
Tomato[8]	0-90	0.034	0.0166	0.0113-0.0216	0.0325	0.0258-0.0387	0.0517	0.0438-0.0608	3.34	2.58-4.09

ND- Not determined. NC- Not calculable.

*Endpoints and/or confidence limits are outside the tested range of concentrations and should be interpreted cautiously.

[1] Significant decrease in cucumber survival, inhibition of 34, 72 and 100% at the 0.067, 0.13 and 0.27 lb ai/A treatments, respectively, compared to the negative control (Williams test, $p<0.05$).

[2] Significant decrease in oilseed rape survival, inhibition of 68, 95 and 100% at the 0.034, 0.067 and 0.13 lb ai/A treatments, respectively, compared to the negative control (Jonckheere-Terpstra Step-Down test, $p<0.05$).

[3] Significant decrease in radish survival, inhibition of 21, 33, 92, 100 and 100% at the 0.017, 0.034, 0.067, 0.13 and 0.27 lb ai/A treatments, respectively, compared to the negative control (Jonckheere-Terpstra Step-Down test, $p<0.05$).

[4] Significant decrease in ryegrass survival, inhibition of 38 and 100% at the 0.034 and 0.067 lb ai/A treatments, respectively, compared to the negative control (Jonckheere-Terpstra Step-Down test, $p<0.05$).

[5] Significant decrease in sorghum survival, inhibition of 55 and 87% at the 0.034 and 0.067 lb ai/A treatments, respectively, compared to the negative control (Williams test, $p<0.05$).

[6] Significant decrease in sugarbeet survival, inhibition of 15 to 100% from the 0.0042 to the 0.067 lb ai/A treatments compared to the negative control (Jonckheere-Terpstra Step-Down test, $p<0.05$).

[7] Significant decrease in sunflower survival, inhibition of 100 and 100% at the 0.27 and 0.53 lb ai/A treatments compared to the negative control (Mann-Whitney U Two-Sample test, $p<0.05$).

[8] Significant decrease in tomato survival, inhibition of 56, 91, 100 and 100% at the 0.067, 0.13, 0.27 and 0.53 lb ai/A treatments, respectively, compared to the negative control (Jonckheere-Terpstra Step-Down test, $p<0.05$).

Mid-study emergence											
Control	Cucumber	Oat	Oilseed Rape	Radish	Ryegrass	Sorghum	Soybean	Sugarbeet	Sunflower	Tomato	Formulation Blank
Not reported											N/A

Plant Injury Index*											
Control	Cucumber	Oat	Oilseed Rape	Radish	Ryegrass	Sorghum	Soybean	Sugarbeet	Sunflower	Tomato	Formulation Blank
0	0-E*	0-D	0-E*	0-E*	0-E*	0	0-D	0-E*	0-B*	0-A*	N/A

0=No injury or effect; A=Slight symptoms throughout the whole plant or more moderate symptoms on a small area (i.e. one leaf); B=Moderate symptoms throughout the whole plant or severe symptoms on limited area, (i.e.one-two leaves); C=Severe symptoms throughout whole plant with younger or newly developed leaves growing normally; D=Total plant symptoms with the plant showing poor vigor; E=Moribund.
*No data at the highest treatment level(s) due to >90% mortality.

Flufenacet

Monocot

Most sensitive monocot: Ryegrass, based on dry weight

EC_{50}/IC_{50}: 0.0217 lb ai/A — 95% C.I.: 0.0138-0.0342 lb ai/A
EC_{25}/IC_{25}: 0.00338 lb ai/A — 95% C.I.: 0.00157-0.00643 lb ai/A
EC_{05}/IC_{05}: 0.000232 lb ai/A — 95% C.I.: N/A-0.00133 lb ai/A
NOEC: 0.0023 lb ai/A
Slope: N/A — 95% C.I.: N/A

Dicot

Most sensitive dicot: Radish, based on dry weight*

EC_{50}/IC_{50}: 0.0187 lb ai/A — 95% C.I.: 0.0121-0.0289 lb ai/A
EC_{25}/IC_{25}: 0.00505 lb ai/A — 95% C.I.: 0.00124-0.0126 lb ai/A
EC_{05}/IC_{05}: 0.000767 lb ai/A — 95% C.I.: N/A-0.00445 lb ai/A
NOEC: 0.0084 lb ai/A
Slope: N/A — 95% C.I.: N/A

*IC_{25} is less than the NOAEC; seedling growth and the trends analyses may have been affected by 92-100% mortality at the three highest treatment levels.

Isoxaflutole

Monocot

Most sensitive monocot: Ryegrass, based on dry weight

EC_{50}/IC_{50}: 0.0026 lb ai/A — 95% C.I.: 0.0017-0.0042 lb ai/A
EC_{25}/IC_{25}: 0.00041 lb ai/A — 95% C.I.: 0.00019-0.00078 lb ai/A
EC_{05}/IC_{05}: 0.000028 lb ai/A — 95% C.I.: N/A-0.00016 lb ai/A
NOEC: 0.00028 lb ai/A
Slope: N/A — 95% C.I.: N/A

Dicot

Most sensitive dicot: Radish, based on dry weight*

EC_{50}/IC_{50}: 0.0023 lb ai/A	95% C.I.: 0.0015-0.0035 lb ai/A
EC_{25}/IC_{25}: 0.00062 lb ai/A	95% C.I.: 0.00015-0.0015 lb ai/A
EC_{05}/IC_{05}: 0.000093 lb ai/A	95% C.I.: N/A-0.00054 lb ai/A
NOEC: 0.00102 lb ai/A	
Slope: N/A	95% C.I.: N/A

*IC_{25} is less than the NOAEC; seedling growth and the trends analyses may have been affected by 92-100% mortality at the three highest treatment levels.

D. STUDY DEFICIENCIES:

1. Survival was the most sensitive endpoint for sorghum, sugarbeet and sunflower, with additional significant effects on survival in all other species except oat and soybean. This study is designed to capture sub-lethal effects, therefore survival is not expected to be the most sensitive endpoint and low survivorship may have confounded seedling growth effects in all species except oat and soybean.

2. The IC_{25} for the most sensitive dicot radish, based on dry weight, was less than the NOAEC. Seedling growth and the trends analyses for radish may have been affected by 92 to 100% mortality at the three highest treatment levels. Radish was retained as the most sensitive endpoint for dicots because radish dry weight was significantly inhibited at lower concentrations; significant inhibitions in radish dry weight were over 60% at the 0.017, 0.034 and 0.067 lb ai/A treatment levels. There was 100% mortality of radish at the two highest treatment levels, 0.13 and 0.27 lb ai/A.

3. Three monocots were studied and OCSPP guidelines recommend including four monocots in the study.

4. Mean seedling control survival was 80% for cucumber, 78% for sorghum and 80% for sunflower; OCSPP recommends that mean control seedling survival be at least 90% at test termination.

5. Seedling height was not measured in the study.

6. The physico-chemical properties of the test material were not reported.

7. Soil CEC and % soil moisture were not reported. Water source and volume applied not reported.

8. It was not stated if this study was conducted according to FIFRA Good Laboratory Practice Standards as published by the U.S. EPA, 40 CFR Part 160 (1989).

E. REVIEWER'S COMMENTS:

The reviewer and study author results were in agreement for the most sensitive monocot and dicot species based on the IC_{25}/EC_{25}. Note: in the study author's report, the most sensitive species was based on the EC/IC_{50} and was sugarbeet based on survival. Based on the EC/IC_{25}, the study author's most sensitive monocot was ryegrass based on dry weight, with NOAEC and IC_{25} values of 0.0023 and 0.0039 lb ai/A, respectively, and the reviewer's most sensitive monocot was also ryegrass based on dry weight, with NOAEC and IC_{25} values of 0.0023 and 0.00338 lb ai/A, respectively. Based on the EC/IC_{25}, the study author's most sensitive dicot was radish based on dry weight, with NOAEC and IC_{25} values of 0.0084 and 0.0059 lb ai/A, respectively, and the reviewer's most sensitive monocot was also radish based on dry

weight, with NOAEC and IC_{25} values of 0.0084 and 0.00505 lb ai/A, respectively.

The IC_{25} for the most sensitive dicot radish, based on dry weight, was less than the NOAEC. Seedling growth and the trends analyses for radish may have been affected by 92 to 100% mortality at the three highest treatment levels. Dry weight significant inhibitions in radish were over 60% at the 0.017, 0.034 and 0.067 lb ai/A treatment levels, and as a result, radish was retained as the most sensitive dicot. There was 100% mortality of radish at the two highest treatment levels, 0.13 and 0.27 lb ai/A.

The reviewer's results are presented in the Executive Summary and Conclusions sections of this DER.

The study author used shattercane and sorghum (*sorghum vulgare*) interchangeably, but for the purposes of this report, the reviewer used only the species term sorghum (emergence 83%).

The in-life portion of this study was initiated on August 24, 2005 and completed on December 22, 2005.

F. CONCLUSIONS:

This study is scientifically sound and is classified as acceptable. The most sensitive monocot species was ryegrass based on dry weight, with NOAEC and IC_{25} values of 0.0023 and 0.00338 lb ai/A, respectively; and the most sensitive dicot species was radish based on dry weight, with NOAEC and IC_{25} values of 0.0084 and 0.00505 lb ai/A, respectively. In terms of the other active ingredient Isoxaflutole, the NOAEC and IC_{25} values for ryegrass based on dry weight were 0.00028 and 0.00041 lb ai/A Isoxaflutole, respectively, and the NOAEC and IC_{25} values for radish based on dry weight were 0.0010 and 0.00062 lb ai/A Isoxaflutole, respectively.

Based on reviewer calculated total formulation concentrations, the NOAEC and IC_{25} values for ryegrass based on dry weight were 0.0064 and 0.0094 lb/A total formulation, respectively, and the NOAEC and IC_{25} values for sugarbeet based on survival were 0.0233 and 0.014 lb/A total formulation, respectively.

Flufenacet
Most sensitive monocot and IC_{25}: Ryegrass (dry weight, 0.00338 lb ai/A Flufenacet).
Most sensitive dicot and IC_{25}: Radish (dry weight, 0.00505 lb ai/A Flufenacet).

Isoxaflutole
Most sensitive monocot and IC_{25}: Ryegrass (dry weight, 0.00041 lb ai/A Isoxaflutole).
Most sensitive dicot and IC_{25}: Radish (dry weight, 0.00062 lb ai/A Isoxaflutole).

Total formulation
Most sensitive monocot and IC_{25}: Ryegrass (dry weight, 0.0094 lb/A Total Formulation).
Most sensitive dicot and IC_{25}: Radish (dry weight, 0.014 lb/A Total Formulation).

III. REFERENCES:

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5. DIN 19682-2 (Marz 1973) Bodenuntersuchungsverfahren im landwirtschaftlichen Wasserbau, Felduntersuchungen. Ermittlung der Bodenart.

6. OECD (Draft Document July 2000): Guideline for the testing of chemicals, Proposal for updating Guideline 208. Terrestrial (Non-Target) Plant Test: 208 A: Seedling Emergence and Seedling Growth Test, 208 B: Vegetative Vigour Test."

7. THE OECD PRINCIPLES OF GOOD LABORATORY PRACTICE, adopted by Council on 26th November 1997 [C(97)186/Final], Environment Directorate, Organisation for Economic Co-operation and Development, Paris 1998.

8. Ratte, M. ToxRat Professional (version 2.09, release 27.01.2005) ToxRat Solutions GmbH, Naheweg 15, 52477 Alsdorf.